



## New designer drug 4'-methyl- $\alpha$ -pyrrolidinohexanophenone: studies on its metabolism and toxicological detection in urine using gas chromatography–mass spectrometry

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### Abstract

*R,S*-4'-Methyl- $\alpha$ -pyrrolidinohexanophenone (MPHP) is a new designer drug which has appeared on the illicit drug market. The aim of this study was to identify the MPHP metabolites using solid-phase extraction, ethylation or acetylation, as well as to develop a toxicological detection procedure in urine using solid-phase extraction, trimethylsilylation and GC–MS. Analysis of urine samples of rats treated with MPHP revealed that MPHP was completely metabolized by hydroxylation of the tolyl methyl group followed by dehydrogenation to the corresponding carboxylic acid, hydroxylation of the side chain, hydroxylation of the pyrrolidine ring with subsequent dehydrogenation to the corresponding lactam and/or reduction of the keto group. The carboxy and/or hydroxy groups were found to be only partly conjugated. Based on these data, MPHP could be detected in urine via its metabolites by GC–MS using mass chromatography for screening and library search for identification.

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**Keywords:** Designer drug; Metabolism; 4'-Methyl- $\alpha$ -pyrrolidinohexanophenone

### 1. Introduction

$\alpha$ -Pyrrolidinopropiophenone derivatives like *R,S*- $\alpha$ -pyrrolidinopropiophenone (PPP) itself, *R,S*-4'-methyl- $\alpha$ -pyrrolidinopropiophenone (MPPP), *R,S*-4'-methoxy- $\alpha$ -pyrrolidinopropiophenone (MOPPP) and *R,S*-3',4'-methylenedioxy- $\alpha$ -pyrrolidinopropiophen-

one (MDPPP) are new designer drugs which have appeared on the illicit drug market [1–4]. These substances with the exception of MOPPP are scheduled in the German Controlled Substances Act and possession is strictly prohibited. Elongating the side chain of the PPPs by three methylene groups leads to the formation of  $\alpha$ -pyrrolidinohexanophenones (PHPs), of which *R,S*-4'-methyl- $\alpha$ -pyrrolidinohexanophenone (MPHP) was the first to have been seized. It is a homologue of MPPP, whose metabolism and toxicological detection has already been described [4]. So far, little information about

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the dosage or the pharmacological and toxicological effects of MPHP is available. The chemical structures of all the  $\alpha$ -pyrrolidinophenones are closely related to  $\alpha$ -aminopropiophenone anorectics like amfepramone, drugs of abuse like cathinone/methcathinone and antidepressants like bupropion, and might therefore evoke similar effects including dopamine release and sympathomimetic properties [5–8]. The metabolism of MPHP has not yet been studied. However, knowledge of metabolic steps is a prerequisite for developing toxicological screening procedures and for toxicological risk assessment, as in both cases the metabolites may play a major role. So far, procedures for determination of or screening for MPHP and/or its metabolites have not yet been published.

The aim of the presented study was firstly to identify the MPHP metabolites in rat urine using GC–MS in the electron impact (EI) and positive-ion chemical ionization (PICI) mode and secondly to develop a toxicological screening procedure based on the identified metabolites using EI GC–MS.

## 2. Experimental

### 2.1. Chemicals and reagents

All chemicals used were obtained from E. Merck, Darmstadt (Germany) and were of analytical grade. *R,S*-MPHP- $\text{HNO}_3$  was provided from Hessisches Landeskriminalamt, Wiesbaden (Germany) for research purposes.

### 2.2. Urine samples

The investigations were performed using urine of male rats (Wistar, Charles River, Sulzfeld, Germany) which were administered a single 20-mg/kg body mass dose (for metabolism studies) or a 1-mg/kg body mass dose (for development of the screening procedure) of MPHP in an aqueous suspension by gastric intubation. Urine was collected separately from the faeces over a 24-h period. All samples were directly analyzed and then stored at  $-20^\circ\text{C}$  until further analysis. Blank urine samples were collected before drug administration to check whether the samples were free of interfering compounds.

### 2.3. Sample preparation for metabolism studies

A 0.5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 *M*) and incubated at  $37^\circ\text{C}$  for 12 h with 50  $\mu\text{l}$  of a mixture (100 000 Fishman units per ml) of glucuronidase (EC 3.2.1.31) and arylsulfatase (EC 3.1.6.1). The urine sample was then diluted with 2.5 ml of water and loaded on an Isolute Confirm HXC cartridge (130 mg, 3 ml), previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was washed with 1 ml of water and 1 ml of 0.01 *M* hydrochloric acid. The retained non-basic compounds were first eluted into 1.5-ml reaction vials with 1 ml of methanol (fraction 1), whereas the basic compounds were eluted in a second step into a different vial with 1 ml of a freshly prepared mixture of methanol/aqueous ammonia (98:2, v/v; fraction 2). The eluates were gently evaporated to dryness under a stream of nitrogen at  $56^\circ\text{C}$  and derivatized by ethylation according to Ref. [4] or acetylation according to Ref. [9]. Briefly, ethylation was performed after reconstitution in 50  $\mu\text{l}$  of methanol and 50  $\mu\text{l}$  of a solution of diazoethane in diethyl ether, and synthesized according to the procedure of McKay et al. [10], and the reaction vials sealed and left at room temperature for 8 h. Thereafter, the mixture was once again gently evaporated to dryness under a stream of nitrogen, and redissolved in 100  $\mu\text{l}$  of methanol. Acetylation was conducted with 100  $\mu\text{l}$  of an acetic anhydride–pyridine mixture (3:2, v/v) for 5 min under microwave irradiation at  $\sim 440\text{ W}$  [9,11,12]. After evaporation, the residue was dissolved in 100  $\mu\text{l}$  of methanol. A 3- $\mu\text{l}$  aliquot was injected into the GC–MS. The same procedure with the exception of enzymatic hydrolysis was used to study which metabolites of MPHP are excreted as glucuronides and/or sulfates.

### 2.4. Sample preparation for toxicological analysis

A 0.5-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 *M*) and incubated at  $56^\circ\text{C}$  for 1 h with 50  $\mu\text{l}$  of a mixture of glucuronidase and arylsulfatase (same as used in Section 2.3). This sample was diluted with 2.5 ml of water and loaded on an Isolute Confirm HXC cartridge (130 mg,

3 ml), previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was washed with 1 ml of water, 1 ml of 0.01 M hydrochloric acid and 1 ml of methanol. The retained compounds were then eluted into a 1.5-ml reaction vial with 1 ml of a freshly prepared mixture of methanol/aqueous ammonia (98:2, v/v). The eluate was gently evaporated to dryness under a stream of nitrogen at 56 °C and then reconstituted in 50 µl of ethyl acetate and silylated after addition of 50 µl *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) for 5 min under microwave irradiation at 440 W. A 2-µl aliquot of this mixture was injected into the GC with an alcohol- and water-free syringe.

### 2.5. Gas chromatography–mass spectrometry

The MPHP metabolites were separated and identified in derivatized urine extracts using a Hewlett-Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m×0.2 mm I.D.), cross linked methylsilicone, 330-nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate, 1 ml/min; column temperature, programmed from 100 to 310 °C at 30°/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full scan mode, *m/z* 50–550 u; EI ionization mode: ionization energy, 70 eV; chemical ionization using methane, positive mode (PICI): ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface heated at 260 °C.

For toxicological detection of MPHP and its trimethylsilylated metabolites, mass chromatography with the selected ions *m/z* 140, 178, 221 and 228 was used. Generation of the mass chromatograms could be started by clicking the corresponding pull down menu which executes the user defined macros [13] (the macros can be obtained from the authors: e-mail: hans.maurer@uniklinik-saarland.de). The identity of the peaks in the mass chromatograms was confirmed by computerized comparison [14] of the mass spectra underlying the peaks (after background

subtraction) with reference spectra (Fig. 1, mass spectra Nos. 1, 13–15) recorded during this study.

## 3. Results and discussion

### 3.1. Sample preparation

Cleavage of conjugates by gentle enzymatic hydrolysis was necessary before extraction and GC–MS analysis of the suspected metabolites in order not to overlook conjugated metabolites. As usual for routine screening procedures, incubation was performed at a higher temperature and for a shorter time in contrast to the metabolism study, in which an almost complete cleavage should be achieved and temperature stress should be avoided. The authors' common liquid–liquid extraction under alkaline conditions followed by acetylation [9,15–18] was not appropriate, because most of the metabolites showed amphoteric properties. In addition, volatility of the free bases and the instability of the analytes under alkaline and high temperature conditions caused difficulties [19,20]. In contrast, solid-phase extraction (SPE) showed good results because mixed-mode SPE has proven to be suitable for the extraction of amphoteric compounds.

Derivatization was needed for sensitive detection of metabolites after lower drug doses. For metabolism studies, acetylation was preferred for derivatization of metabolites with primary and secondary amino groups as well as of alcoholic and/or phenolic hydroxy groups [15,21,22]. Ethylation was preferred for derivatization of metabolites with carboxy groups [4]. In addition, ethylation by diazoethane has the further advantage that phenolic hydroxy groups can be derivatized in contrast to alcoholic groups, allowing both types of hydroxy groups to be distinguished from each other. Moreover, ethylation is favored over diazomethane methylation, because it allows metabolic methylation and derivatization to be distinguished from each other.

However, for the toxicological detection procedure, common trimethylsilylation was preferred. In routine work, trimethylsilylation is safer and easier to handle and the reagent is commercially available. All expected target analytes in urine after intake of

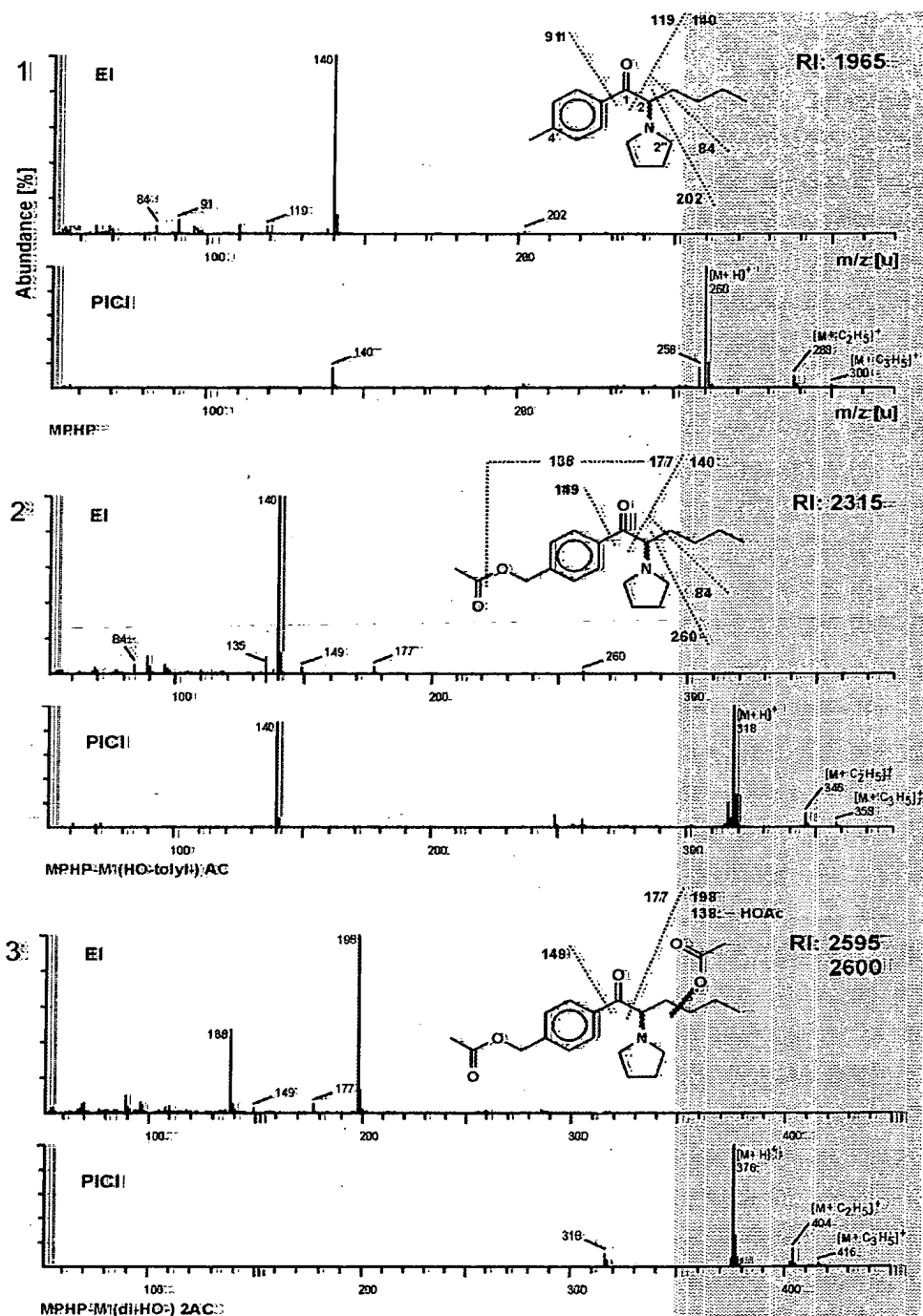


Fig. 1. EI and PICI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of MPHP and its metabolites after ethylation, acetylation or trimethylsilylation. The axes are only labelled for 1.

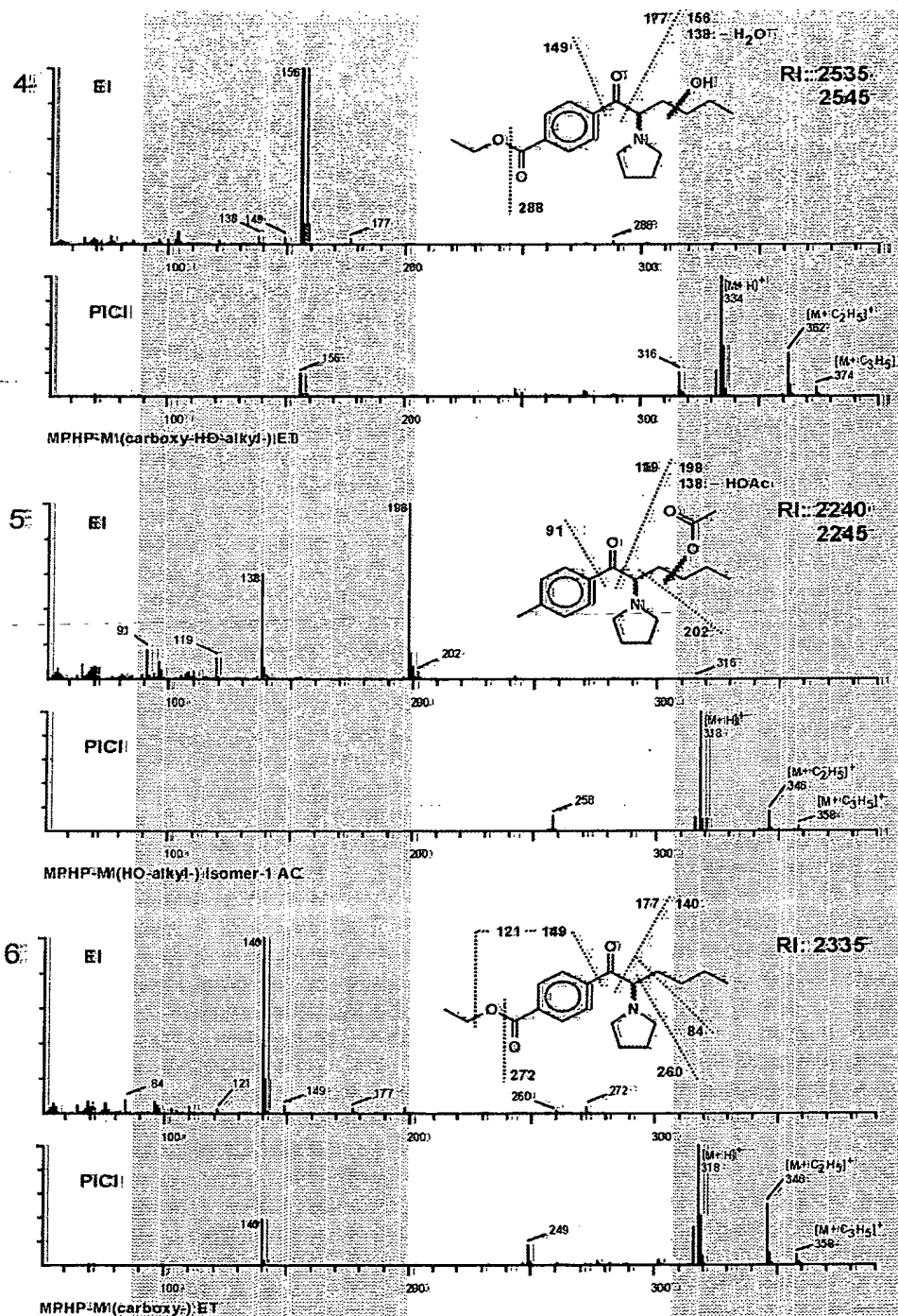


Fig. 1. (continued)

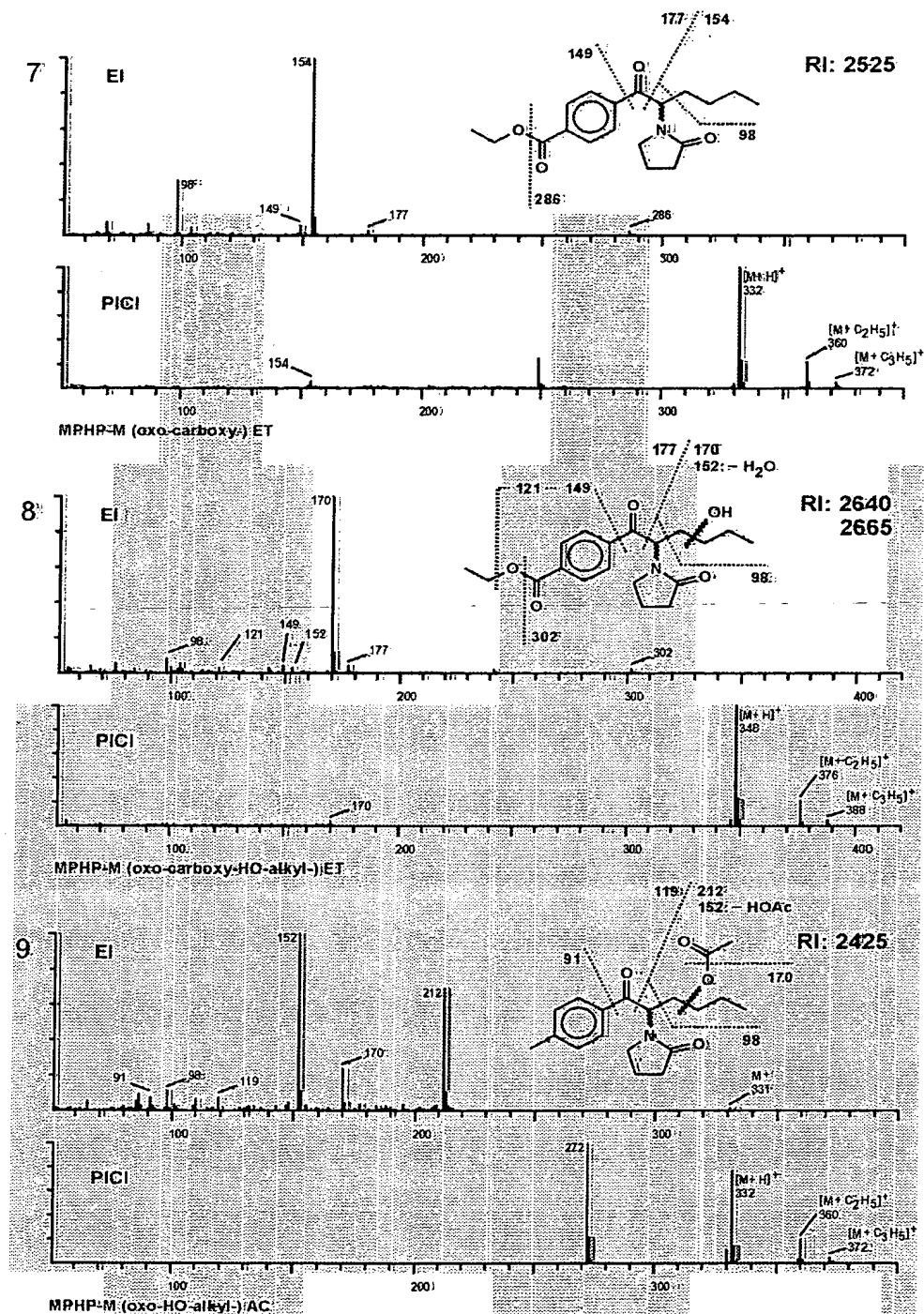


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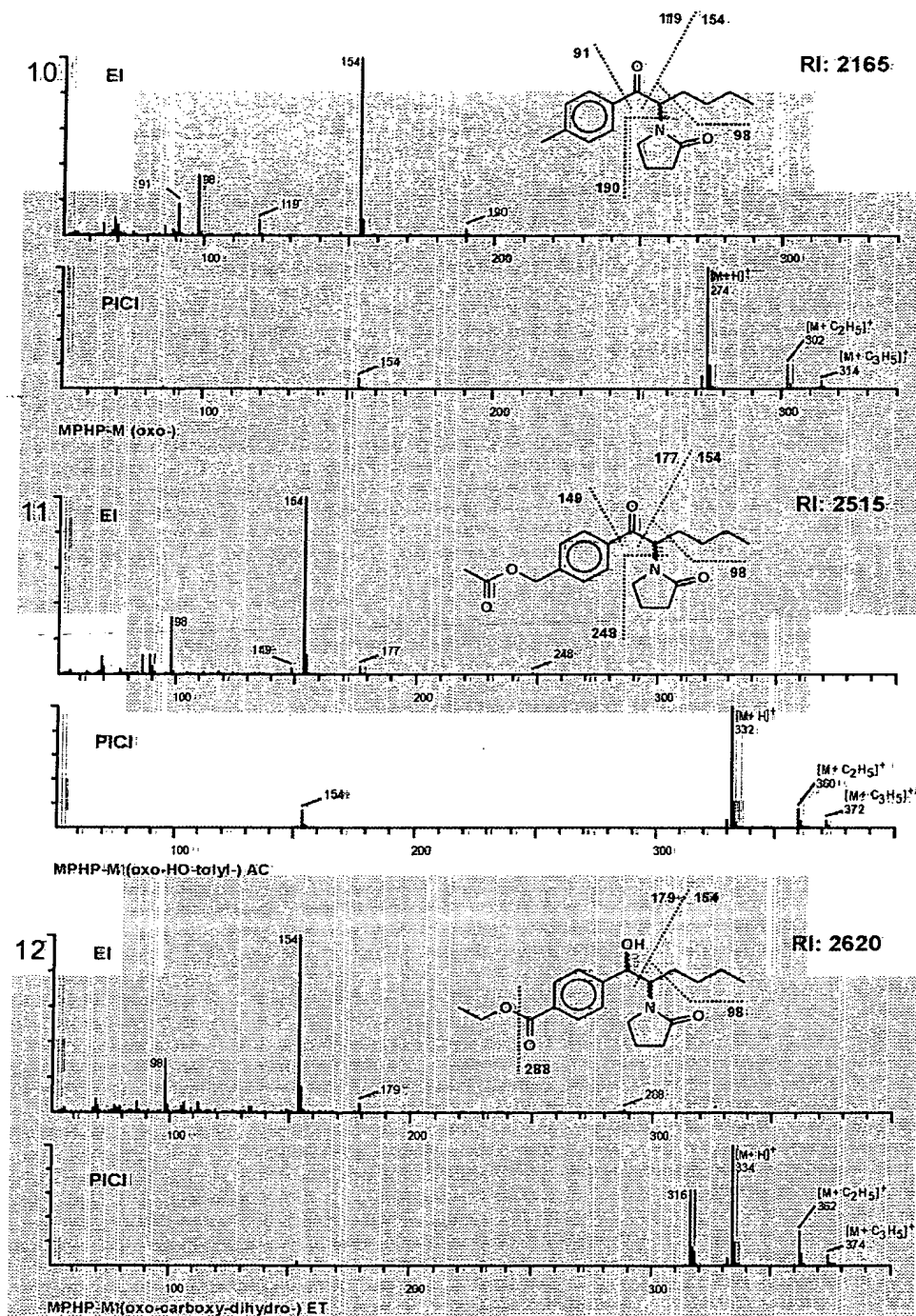


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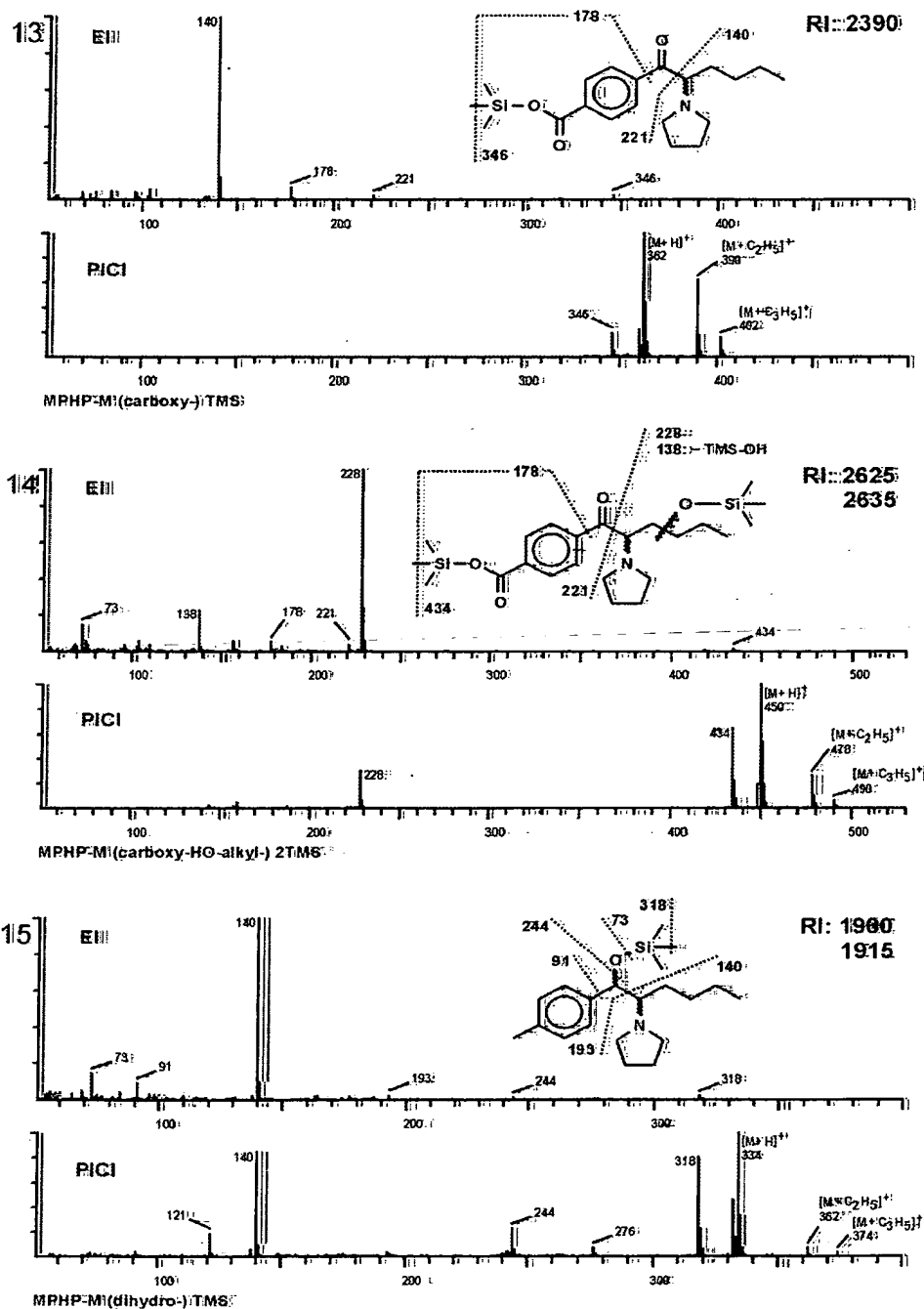


Fig. 1. (continued)



MPHP showed good GC properties after trimethylsilylation.

### 3.2. Identification of metabolites

The urinary metabolites of MPHP were identified by EI and PICI MS after GC separation. The postulated structures of the metabolites were deduced from the fragments detected in the EI mode which were interpreted in correlation to those of the parent compound according to the rules described by McLafferty and Turecek [23]. In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded, because they contain strong molecular peaks ( $M+H$ ) with adduct ions typical for PICI using methane as reagent gas.

EI and PICI mass spectra, the gas chromatographic retention indices (RI), structures and predominant fragmentation patterns of MPHP and its ethylated, acetylated or trimethylsilylated metabolites are shown in Fig. 1. Only one mass spectrum each of the isomeric compounds (mass spectra Nos. 3–5, 8, 14 and 15) is shown in order to save space. The spectra are very similar, so that one can be used for identification of both peaks considering the given two different retention indices. However, it could not be differentiated under the applied conditions, whether these isomers are the corresponding diastereomers or positional isomers concerning the hydroxy group in the side chain. If the two peaks represent the positional isomers, each diastereomer of both positional isomers would not have been separated. The potential positional isomers or diastereomers of compound 9 as well as the diastereomers of compound 12 could not be separated under the applied conditions.

The following metabolites of MPHP (mass spectrum No. 1 in Fig. 1) could be identified after high dose application: 4'-hydroxymethyl-side-chain-hydroxy-PHP (mass spectrum No. 3), 4'-carboxy-side-chain-hydroxy-PHP (mass spectrum No. 4), 4'-carboxy-PHP (mass spectrum No. 6), 4'-carboxy-2''-oxo-PHP (mass spectrum No. 7), 4'-carboxy-2''-oxo-side-chain-hydroxy-PHP (mass spectrum No. 8), 2''-oxo-side-chain-hydroxy-MPHP (mass spectrum No. 9), 2''-oxo-MPHP (mass spectrum No. 10), 4'-hydroxymethyl-2''-oxo-PHP (mass spectrum No. 11) and 4'-carboxy-1-dihydro-2''-oxo-PHP (mass spec-

trum No. 12). The latter two metabolites could only be found in extract fraction 1. 4'-Hydroxy-methyl-PHP (mass spectrum No. 2) and side-chain-hydroxy-MPHP (mass spectrum No. 5) are intermediate metabolites which could only be found in rat liver microsomal incubations in extract fraction 2 (for conditions see Ref. [21]).

Many studies in the authors' laboratory demonstrated a high degree of qualitative correspondence of rat and human metabolism [15,16,21,22,24–26]. However, in contrast to the results of the presented rat study on MPHP metabolism, substances like cathinone [19], amfepramone [27] or metamfepramone [28] which are structurally related to MPHP, were shown to be additionally excreted as dihydro metabolites (diastereomers) in humans to a considerable extent. Therefore, the data of dihydro-MPHP (synthesized from MPHP by sodium borohydride hydration according to Ref. [29]) were included in Fig. 1 (mass spectrum No. 15). The GC and MS data of those compounds and derivatives, which are not shown in Fig. 1, will also be included in the forthcoming update of the authors' handbook and library [14,30].

The mass spectra Nos. 3–5, 8 and 9 did not allow identification of the position of the hydroxy group in the side chain. Possible formation of positional isomers has already been discussed above. The mass spectra Nos. 7–12 did not allow identification of the position of the carbonyl group in the pyrrolidine ring. However, as other compounds carrying a pyrrolidine ring are also excreted as their lactam metabolite (e.g. MPPP or nicotine), we postulate that the same happened to MPHP. The parent compound MPHP could not be found, although the limit of detection was as low as 100 ng/ml and the extraction efficiency for MPHP was  $90 \pm 4\%$  ( $n=5$ ).

Based on the identified metabolites of MPHP, the following partly overlapping metabolic pathways could be postulated (Fig. 2): hydroxylation of the tolyl methyl group (Nos. 2, 3 and 11) mostly followed by dehydrogenation to the corresponding carboxy compounds (Nos. 4, 6–8 and 12), hydroxylation of the side chain (leading to diastereomers and possibly to positional isomers, Nos. 3–5, 8 and 9), hydroxylation of the 2''-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam (Nos. 7–12) and/or reduction of the

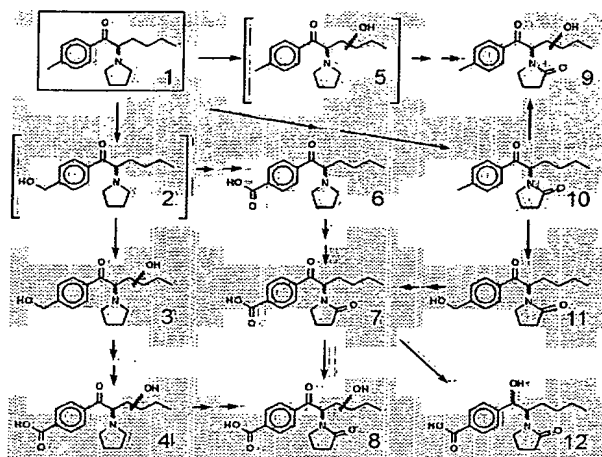


Fig. 2. Proposed scheme for the metabolism of MPHP in rats. Compounds 2 and 5 could only be found after incubation with rat liver microsomes. The numbering of the compounds corresponds to that of the mass spectra of the corresponding derivative in Fig. 1.

keto group to the corresponding secondary alcohol (diastereomers, No. 12). Hydroxylation of the  $\alpha$ -carbon followed by desamination and further biotransformation to the corresponding benzoic acid in analogy to the structurally related amfepramone [27] or pyrrolidinopropiophenones was not observed. As the peaks of the metabolites 3–4, 6–9 and 11–12 were more abundant after enzymatic hydrolysis, it can be concluded that they are partly excreted as glucuronides and/or sulfates.

### 3.3. Toxicological detection by GC–MS

MPHP metabolites were separated by GC and identified by EI-MS after fast enzymatic hydrolysis, SPE and trimethylsilylation. Mass chromatography with the following ions was used to indicate the presence of MPHP and/or its metabolites:  $m/z$  140, 178, 221 and 228. Generation of the mass chromato-

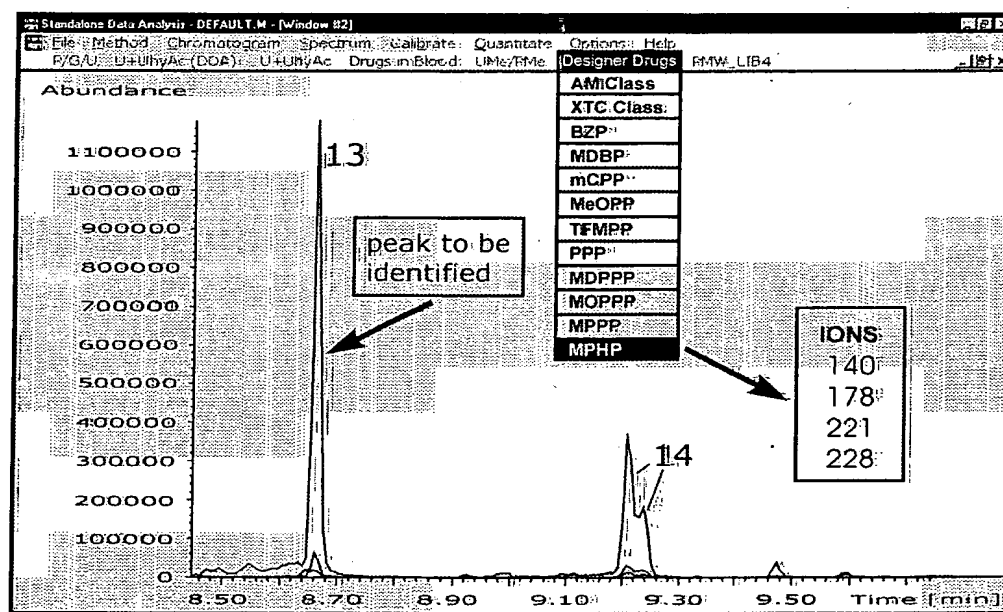


Fig. 3. Typical mass chromatograms with the ions  $m/z$  140, 178, 221 and 228. They indicate the presence of MPHP metabolites in a trimethylsilylated extract of a rat urine sample collected over 24 h after ingestion of 1 mg/kg body mass of MPHP. The numbering of the peaks corresponds to that of the mass spectra of the corresponding derivative in Fig. 1. The merged chromatograms can be differentiated by their colors on a color screen.

grams could be started by clicking the corresponding pull down menu which executes the user defined macros.

The selected ion  $m/z$  140 was used for monitoring the presence of compounds with unchanged pyrrolidine ring and non-hydroxylated side chain (mass spectra Nos. 1, 13, 15 in Fig. 1),  $m/z$  178 and 221 for trimethylsilylated 4'-carboxy compounds (mass spectra Nos. 13 and 14 in Fig. 1) and  $m/z$  228 for trimethylsilylated compounds with hydroxylated side chain (mass spectra No. 14 in Fig. 1).

Fig. 3 shows reconstructed mass chromatograms indicating the presence of MPHP metabolites in a trimethylsilylated extract of rat urine after administration of 1 mg/kg body mass of MPHP. This dose was chosen as it should roughly correspond to a common dose of abusers, since seized tablets of the

related designer drug PPP contained 40 mg. In addition, the chosen dose is in the same range as that of the structurally related medicament amfepramone. The identity of the peak in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with reference spectra recorded during this study [14]. Fig. 4 shows the mass spectrum underlying the marked peak in Fig. 3, the reference spectrum (No. 13 in Fig. 1), the structure, and the hit list found by computer library search. In the authors' experience, the gas chromatographic retention indices (RI) provide preliminary indications, allow the above mentioned positional isomers and/or diastereomers to be distinguished from each other and may be useful to gas chromatographers without an MS facility. Therefore, the RIs are also given in Fig. 1. They were recorded during

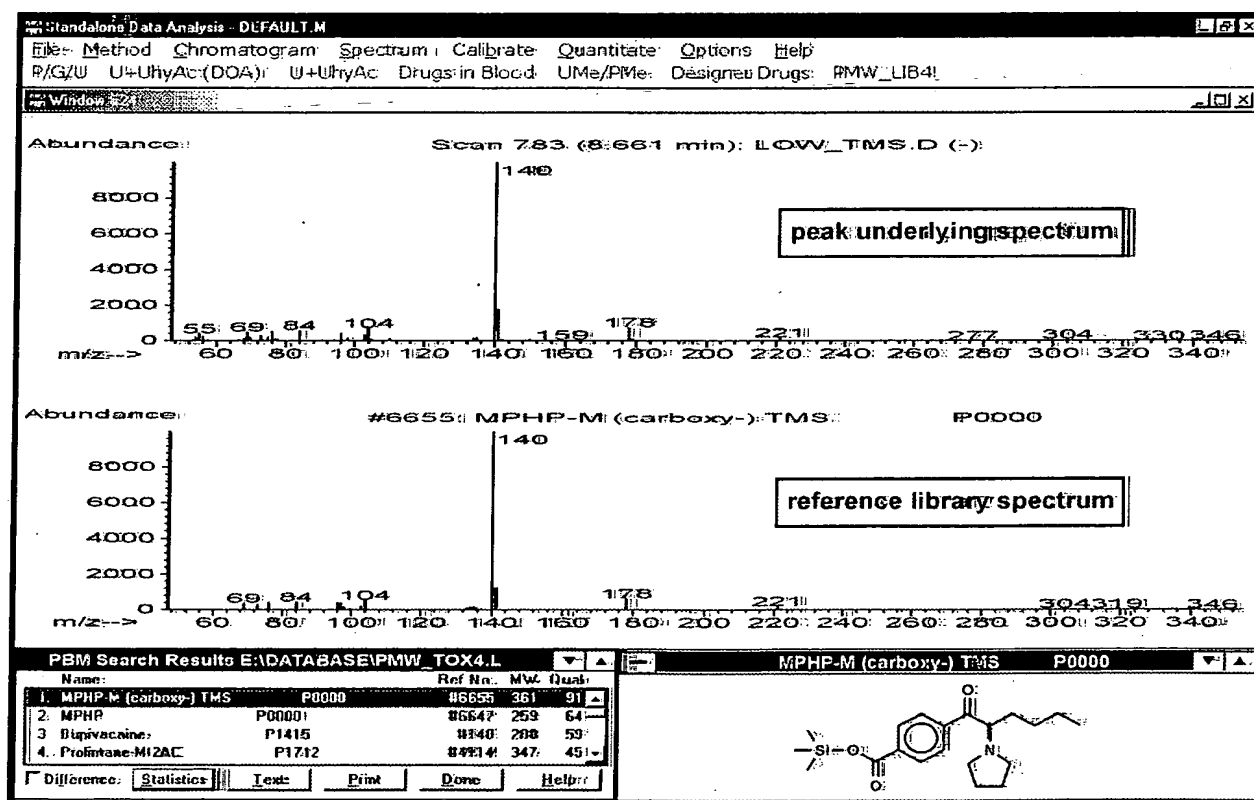


Fig. 4. Mass spectrum underlying the marked peak in Fig. 3, the reference spectrum, the structure, and the hit list found by computer library search.

the GC–MS procedure (Section 2.5) and calculated in correlation with Kovats' indices [31] of the components of a standard solution of typical drugs which is measured daily for testing GC–MS performance [32,33]. The reproducibility of retention indices measured on capillary columns was better using a mixture of drugs than that of the homologous hydrocarbons recommended by Kovats.

Unfortunately, no authentic human urine samples after intake of MPHP were available. However, according to the authors' experience in metabolism and analytical studies on rats and humans [15,16,22], it should be possible to also detect the metabolites found in rat urine, in human urine samples. As already discussed above, the dihydro metabolites (mass spectrum No. 15) might additionally be detectable in human urine. However, as mass  $m/z$  140 would also indicate the presence of dihydro-MPHP and its mass spectrum was included in Fig. 1 and in the library, this metabolite should be detectable in human urine besides the main metabolites found in rats. The extraction efficiency for trimethylsilylated dihydro-MPHP was  $66 \pm 12\%$  and the limit of detection was as low as 5 ng/ml ( $n=5$ ). Having no reference substances for the other metabolites, their extraction efficiencies and limits of detection could not be determined. Extrapolating the rat data, the described screening procedure should be sensitive enough to detect an intake of a common dose of MPHP. The presented screening procedure should also be suitable for other designer drugs of the  $\alpha$ -pyrrolidinopropiophenone type [2–4].

#### 4. Conclusions

The presented studies revealed that the new designer drug MPHP was extensively metabolized. Screening must, therefore, be focussed on metabolites. The described screening procedure should be suitable for detection of MPHP and/or its metabolites in human urine in clinical or forensic cases.

Further studies will show which cytochrome-P450 isoenzymes are involved in the main metabolic steps. This knowledge may allow possible drug–drug interactions and/or toxic risks to be predicted.

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#### References

- [1] P. Roesner, T. Junge, G. Fritschi, B. Klein, K. Thielert, M. Kozlowski, *Toxichem. Krintech.* 66 (1999) 81.
- [2] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, in: F. Pragst, R. Aderjan (Eds.), *Proceedings of the XIIth GTFCh Symposium in Mosbach, Helm, Heppenheim, Germany, 2001*, p. 156.
- [3] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, in: M. Balikova, E. Navakova (Eds.), *Proceedings of the 39th International TIAFT Meeting in Prague, 2001*. Charles University, Prague, 2002., p. 122.
- [4] D. Springer, F.T. Peters, G. Fritschi, H.H. Maurer, *J. Chromatogr. B* 773 (2002) 25.
- [5] M. Martinez, O. Mercado, A. Santamaria, S. Galvan, M. Vazquez, V. Bucio, C. Hall, R. Hernandez, A. Hurtazo, E. Pego, F. Rodriguez, R. Salvatierra, A. Sosa, C. Rios, *Proc. West. Pharmacol. Soc.* 41 (1998) 125.
- [6] R.A. Glennon, M. Yousif, N. Naiman, P. Kalix, *Pharmacol. Biochem. Behav.* 26 (1987) 547.
- [7] P. Kalix, R.A. Glennon, *Biochem. Pharmacol.* 35 (1986) 3015.
- [8] S.G. Bryant, B.G. Guernsey, N.B. Ingram, *Clin. Pharm.* 2 (1983) 525.
- [9] H.H. Maurer, in: K. Pfeleger, H.H. Maurer, A. Weber (Eds.), *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, 2nd ed, Wiley-VCH, Weinheim, 2000, p. 3, Part 4.
- [10] A.F. McKay, W.L. Ott, G.W. Taylor, M.N. Buchanan, J.F. Crooker, *Can. J. Res. (Sec. B)* 28 (1950) 683.
- [11] T. Kraemer, A.A. Weber, H.H. Maurer, in: F. Pragst (Ed.), *Proceedings of the 10th GTFCh Symposium in Mosbach, Helm, Heppenheim, 1997*, p. 200, ISBN 3-923032-10-2.
- [12] H.H. Maurer, J. Bickeboeller-Friedrich, T. Kraemer, F.T. Peters, *Toxicol. Lett.* 112 (2000) 133.
- [13] H.H. Maurer, *Spectroscopy Europe* 6 (1994) 21.
- [14] K. Pfeleger, H.H. Maurer, A. Weber, *Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*, 4th Rev., Agilent Technologies, Palo Alto, CA, 2003 (in preparation).
- [15] R.F. Staack, G. Fritschi, H.H. Maurer, *J. Chromatogr. B* 773 (2002) 35.
- [16] J. Bickeboeller-Friedrich, H.H. Maurer, *Ther. Drug Monit.* 23 (2001) 61.
- [17] H.H. Maurer, *Comb. Chem. High Throughput Screen.* 3 (2000) 461.

- [18] H.H. Maurer, J. Bickeboeller-Friedrich, *J. Anal. Toxicol.* 24 (2000) 340.
- [19] M. Pokrajac, B. Miljkovic, B. Misailovic, *Rapid. Commun. Mass Spectrom.* 5 (1991) 59.
- [20] R. Brenneisen, S. Geissshusler, *Pharm. Acta Helv.* 60 (1985) 290.
- [21] T. Krämer, J. Bickeboeller-Friedrich, H.H. Maurer, *Drug Metab. Dispos.* 28 (2000) 339.
- [22] K. Pfleger, H.H. Maurer, A. Weber, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, 2nd ed, Wiley-VCH, Weinheim, 2000, Part 4.
- [23] F.W. McLafferty, F. Turecek, *Interpretation of Mass Spectra*, University Science Books, Mill Valley, CA, 1993.
- [24] K. Pfleger, H.H. Maurer, A. Weber, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, 2nd ed, VCH, Weinheim, 1992.
- [25] H.H. Maurer, J. Bickeboeller-Friedrich, *Ther. Drug Monit.* 19 (1997) 583.
- [26] T. Krämer, H.H. Maurer, *Ther. Drug Monit.* 24 (2002) 277.
- [27] A.H. Beckett, M. Stanojcic, *J. Pharm. Pharmacol.* 39 (1987) 409.
- [28] S.L. Markantonis, A. Kyroudis, A.H. Beckett, *Biochem. Pharmacol.* 35 (1986) 529.
- [29] K. Schwetlick, *Organikum*, Wiley-VCH, Weinheim, 2001.
- [30] K. Pfleger, H.H. Maurer, A. Weber, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and their Metabolites*, 2nd ed., Part 5, Wiley-VCH, Weinheim, 2003 (in preparation).
- [31] E. Kovats, *Helv. Chim. Acta* 41 (1958) 1915.
- [32] R.A. de-Zeeuw, J.P. Franke, H.H. Maurer, K. Pfleger, *Gas Chromatographic Retention Indices of Toxicologically Relevant Substances and Their Metabolites*, VCH, Weinheim, 1992, report of the DFG Commission for Clinical Toxicological Analysis, special issue of the TIAFT Bulletin.
- [33] DFG Senatskommission für Klinisch-toxikologische Analytik, *J. Clin. Chem. Clin. Biochem.* 20 (1982) 699.